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Exploring the volatile profile of whiskey samples using solid-phase microextraction Arrow and comprehensive two-dimensional gas chromatography-mass spectrometry

Antonio Ferracane[a,b], Natalia Manousi[b,c], Peter Q. Tranchida[a], George A. Zachariadis[c], Luigi Mondello[a,d,e], Erwin Rosenberg[b,*]

[a] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy
[b] Institute of Chemical Technology and Analytics, Vienna University of Technology, Getreidemarkt 9/164, Vienna 1060, Austria
[c] Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece
[d] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy
[e] Department of Sciences and Technologies for Human and Environment, University Campus Bio-Medico of Rome, Rome, Italy

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ABSTRACT

We present a novel sample preparation method for the extraction and preconcentration of volatile organic compounds from whiskey samples prior to their determination by comprehensive two-dimensional gas chromatography (GC × GC) coupled to mass spectrometry (MS). Sample preparation of the volatile compounds, important for the organoleptic characteristics of different whiskeys and their acceptance and liking by the consumers, is based on the use of the solid-phase microextraction (SPME) Arrow. After optimization, the proposed method was compared with conventional SPME regarding the analysis of different types of whiskey (i.e., Irish whiskey, single malt Scotch whiskey and blended Scotch whiskey) and was shown to exhibit an up to a factor of six higher sensitivity and better repeatability by a factor of up to five, depending on the compound class. A total of 167 volatile organic compounds, including terpenes, alcohols, esters, carboxylic acids, ketones, were tentatively-identified using the SPME Arrow technique, while a significantly lower number of compounds (126) were determined by means of conventional SPME. SPME Arrow combined with GC × GC-MS was demonstrated to be a powerful analytical tool for the exploration of the volatile profile of complex samples, allowing to identify differences in important flavour compounds for the three different types of whiskey investigated.

1. Introduction

Whiskey is a type of distilled alcoholic beverage produced from fermented grain mash and it is considered to be one of the most popular alcoholic beverages worldwide [1]. For the production of whiskey, ground cereals and/or malt are mixed with water to produce a mash that is further fermented with yeast. Subsequently, the resulting mixture is distilled to produce a distilled spirit that is finally stored in barrels [2]. Typically, wooden casks produced from charred white oak are employed for the aging process of the final product [1]. The volatile profile of distilled spirits depends on the raw materials used for their production, their manufacturing procedure (i.e., fermentation, distillation, and storage) and their aging process [3]. Whiskey contains a high number of volatile organic compounds (VOCs) that contribute to its aroma and the most abundant among them are esters and alcohols. Other compounds that contribute to the overall aroma of whiskeys include aldehydes, ketones, furanic compounds, terpenes and sulphur compounds [4]. The volatile composition of distilled spirits is directly associated with their acceptance by the consumers. Thus, the determination of VOCs in alcoholic beverages is of the utmost importance for the evaluation of their quality and their safety and for the understanding of their sensory properties [3,5,6].

One-dimensional gas chromatography hyphenated to a mass spectrometer (GC-MS) or to an olfactometric detector are two well-established analytical techniques for the determination of aroma compounds in complex food samples [7,8]. However, the application of these techniques for the analysis of complex food samples, containing a plethora of VOCs, can result in insufficient separation and co-elution of the target analytes due to sheer sample complexity [9]. To overcome these potential drawbacks, comprehensive

* Corresponding author.
E-mail address: erosen@mail.zserv.tuwien.ac.at (E. Rosenberg).

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two-dimensional gas chromatography (GC × GC) can be employed. In GC × GC, analytes are typically separated using a conventional polar or non-polar column, followed by a micro-bore capillary column of different polarity [9]. For this purpose, a modulator (transfer device) is used for trapping and re-injecting the eluent from the exit of the primary column to the head of the second column within some milliseconds [9,10]. Among the different types of GC × GC systems, GC × GC equipped with cryogenic modulators are typically preferred, since they offer the advantages of enhanced sensitivity [9]. Additionally, GC × GC coupled to mass spectrometry (GC × GC–MS) forms a powerful analytical tool for the profiling and fingerprinting of food and beverage VOCs [11].

Currently, the exploration of opportunities of novel green microextraction protocols combined with GC × GC is considered to be an important step towards the development of more environmentally-friendly methodologies and towards the simplification of complex workflows [10]. In this context, solid-phase microextraction (SPME), proposed by Pawliszyn in the early 1990s [12], is until now the most explored format of microextraction technique coupled to both one-dimensional GC, as well as heartcut- and comprehensive two-dimensional GC [10]. SPME exhibits a plethora of benefits including ease of automation, reduced number of sample preparation steps and solvent-free nature [13]. However, the utilization of conventional SPME fibers also exhibits some fundamental drawbacks that are associated with poor mechanical durability and low extraction phase volume [14]. More recently, the SPME Arrow was proposed as an alternative sample preparation technique to conventional SPME. In the SPME Arrow approach, extraction of the target analytes takes place using a coated fiber with an Arrow-shaped tip attached to a robust stainless-steel backbone [6]. This technique can overcome the shortcomings of conventional SPME fibers, while it maintains its main benefits. Thus, the SPME Arrow is characterized by good mechanical robustness and enhanced sensitivity due to the higher extraction phase area and volume.

Due to its inherent advantages, the SPME Arrow has already proven to be a versatile analytical technique for the determination of VOCs in a wide variety of environmental, food, herbal and forensic samples [14–17]. Until now, most applications of SPME Arrow have been focused on the analysis of food samples including grape skins [18], brown rice vinegar [5], milk [6], Korean salt–fermented fish sauce [19], soy sauce [20] and fish samples [21]. Recently, the applications of SPME Arrow have been successfully expanded to the analysis of distilled spirits (i.e., Korean Soju liquor [3] and Chinese Baijiu liquor [22]). Thus, this technique can be a promising alternative to already existing conventional methodologies for the determination of VOCs in whiskey samples.

In this study, SPME Arrow combined with GC × GC–MS was employed for the first time for the exploration of the volatile profile of whiskey samples. The main parameters affecting the performance of the microextraction protocol were thoroughly investigated and optimized. Under optimum conditions, the herein proposed protocol was compared with the conventional SPME technique, to assess the difference of this technique in terms of method repeatability and sensitivity. The ability of the proposed method for the determination of molecules that remain undetermined with conventional SPME was also investigated using three different types of whiskey samples (i.e, “blended Scotch whiskey”, “Irish whiskey” and “single malt Scotch whiskey”).

2. Experimental

2.1. Chemicals and reagents

LC-MS CHROMASOLV™ grade methanol was purchased from Honeywell (Riedel-de Haën GmbH, Seelze, Germany). Concentrated H_3PO_4 (85%) and reagent grade KH_2PO_4 were purchased from Sigma-Aldrich (Steinheim, Germany). 3-methyl-3-pentanol (purity 98.0%) was also supplied by Sigma-Aldrich and was used as internal standard (ISTD). A stock solution (2000 mg L^{-1}) of the ISTD was prepared in methanol and was 10-fold diluted to prepare a working ISTD solution at a concentration of 200 mg L^{-1}. Finally, a C_7–C_{30} alkane mixture was purchased from Supelco (Bellefonte, PA, USA) and was employed for the calculation of the linear retention indices.

The carbon wide range (WR)/polydimethylsiloxane (PDMS) SPME Arrow fibers of 1.1 mm outer diameter and 120 µm phase thickness were purchased from Restek Corporation (Bellefonte, PA, USA). A Restek PAL SPME Manual Injection Kit (Restek Corporation, Bellefonte, PA, USA) was also employed for the extraction and the desorption of the VOCs of the whiskey samples. Conventional carboxen (CAR)/PDMS SPME fibers of 75 µm phase thickness were purchased from Supelco (Bellefonte, PA, USA) and they were attached to an SPME fiber holder (Supelco) for the extraction procedure. Prior to the extraction, the SPME Arrow fibers and the conventional SPME fibers were preconditioned in the injector port of the GC system based on the recommendations of the manufacturers. The quality of the conditioning process was confirmed by taking fiber blanks prior to the analysis. All extractions were performed using an IKA® RCT basic magnetic stirrer (IKA Labortechnik, Staufen, Germany).

2.2. Instrumentation

A GC × GC–MS system consisting of a GC-2010 Shimadzu gas chromatograph equipped with a split/splitless injector and a QP2010 Ultra quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan) was used. An Rtx-5MS column 30 m × 0.25 mm ID, 0.25 µm d_f, (Crossbond 5% diphenyl-95% dimethyl polysiloxane) (Restek Corporation, Bellefonte, PA, USA) was used as first dimension and was connected to an uncoated capillary column (1 m × 0.25 mm ID). A dual-stage loop-type cryogenic modulator (Zoex Corporation, Houston, TX) was installed in the GC × GC–MS system and the uncoated tubing was further connected to a Stabilwax®-MS 2 m × 0.15 mm ID, 0.15 µm d_f column (Crossbond Carbowax polyethylene glycol) (Restek Corporation). Helium (99.999%) was employed as carrier gas at 61.8 kPa at the beginning of the analysis (constant linear velocity mode). The injector temperature was set at 280 °C and the split mode was employed as injection mode, at a split ratio of 25:1. The initial oven temperature was 40 °C which was kept constant for 5 min. After this time span, the temperature was raised to 230 °C using a ramp of 5 °C min^{-1} and further increased to 250 °C using a ramp of 50 °C min^{-1}. The total run time was 48.40 min. The working parameters of the cryogenic modulator were the following: modulation period: 4 s, hot jet temperature: 350 °C and hot jet duration: 250 ms.

With regard to the MS system, the scan mode with a mass range of m/z 45–445 was employed. The scan speed of mass analyzer was set at 20,000 amu s^{-1} (33 Hz spectral acquisition frequency). The ionization mode was electron ionization (70 eV), the ion source temperature was 200 °C, while the interface source temperature was 250 °C. System control and data handling were performed using the GCMS solution software ver. 4.5., while the bidimensional chromatograms were generated by using the ChromSquare software ver. 2.3 (Shimadzu Europe, Duisburg, Germany). The tentative identification of the VOCs was carried out by using the “W11N17” (Wiley11-Nist17, Wiley, Hoboken, NJ, USA; Mass Finder 3) and “FFNSC 4.0” (Shimadzu Europa GmbH, Duisburg, Germany) mass spectral libraries. The use of linear retention indices in GC × GC was applied as previously explored by Purcaro [23]. Regarding the use of LRIs and mass spectra similarity,

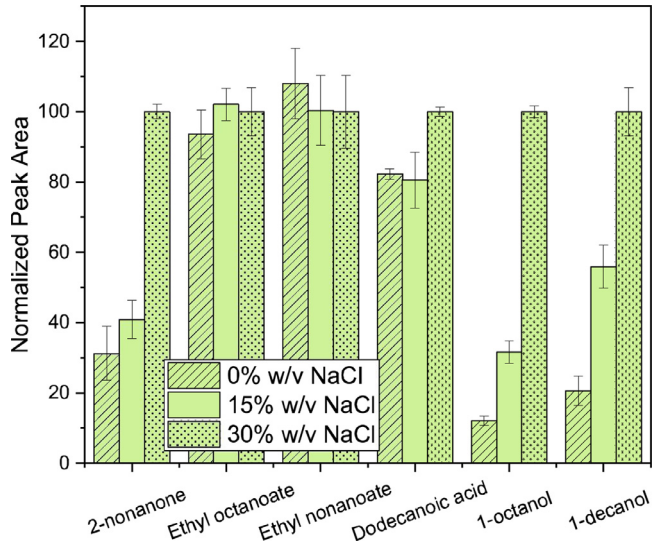

Fig. 1. Evaluation of different NaCl concentrations ($n = 3$). Sample volume: 35 mL, ethanol concentration: 12% v/v, pH: 3.3, adsorption time: 45 min, stirring rate: 500 rpm.

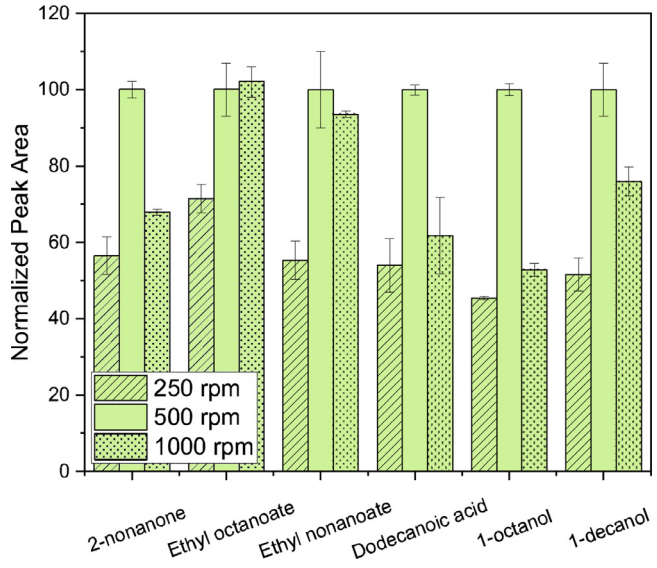

Fig. 2. Evaluation of different stirring rates ($n = 3$). Sample volume: 35 mL, ethanol concentration: 12% v/v, pH: 3.3, adsorption time: 45 min, NaCl content: 30% w/v.

a matching interval of ± 20 and a similarity value of at least 80% were applied, respectively.

2.3. Sample collection

In this study, three different types of whiskey samples, namely "blended Scotch whiskey", "Irish whiskey" and "single malt Scotch whiskey" were collected from the local market of Vienna, Austria, and analyzed. Before their analysis, all samples were stored in the dark at ambient temperature.

2.4. Extraction of VOCs from whiskey samples

Prior to the determination of the VOCs of whiskey samples, the samples were diluted with 25 mmol L^{-1} phosphate buffer (pH 3.3) to obtain a final ethanol content of 12% v/v [24]. For the SPME Arrow procedure, an aliquot of 35 mL of the diluted sample was placed in a 50 mL glass (headspace) vial. The sample was saturated

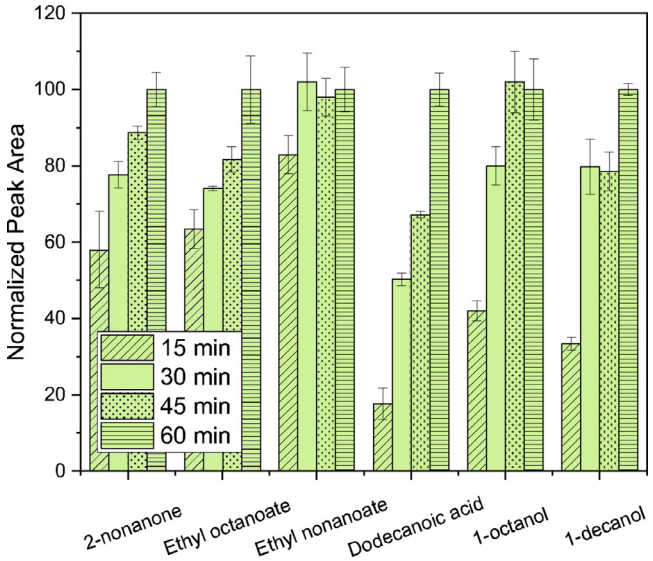

Fig. 3. Evaluation of different extraction times ($n = 3$). Sample volume: 35 mL, ethanol concentration: 12% v/v, pH: 3.3, stirring rate: 500 rpm, NaCl concentration: 30% w/v.

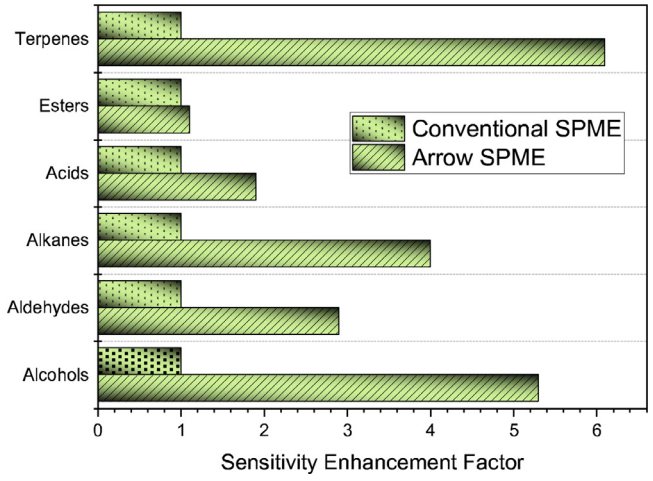

Fig. 4. Comparison of method sensitivity between SPME Arrow and conventional SPME.

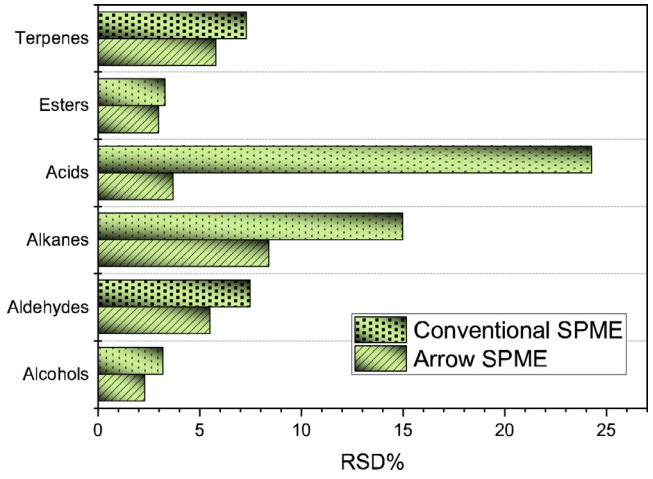

Fig. 5. Comparison of method repeatability between SPME Arrow and conventional SPME techniques for different classes of chemical compounds.

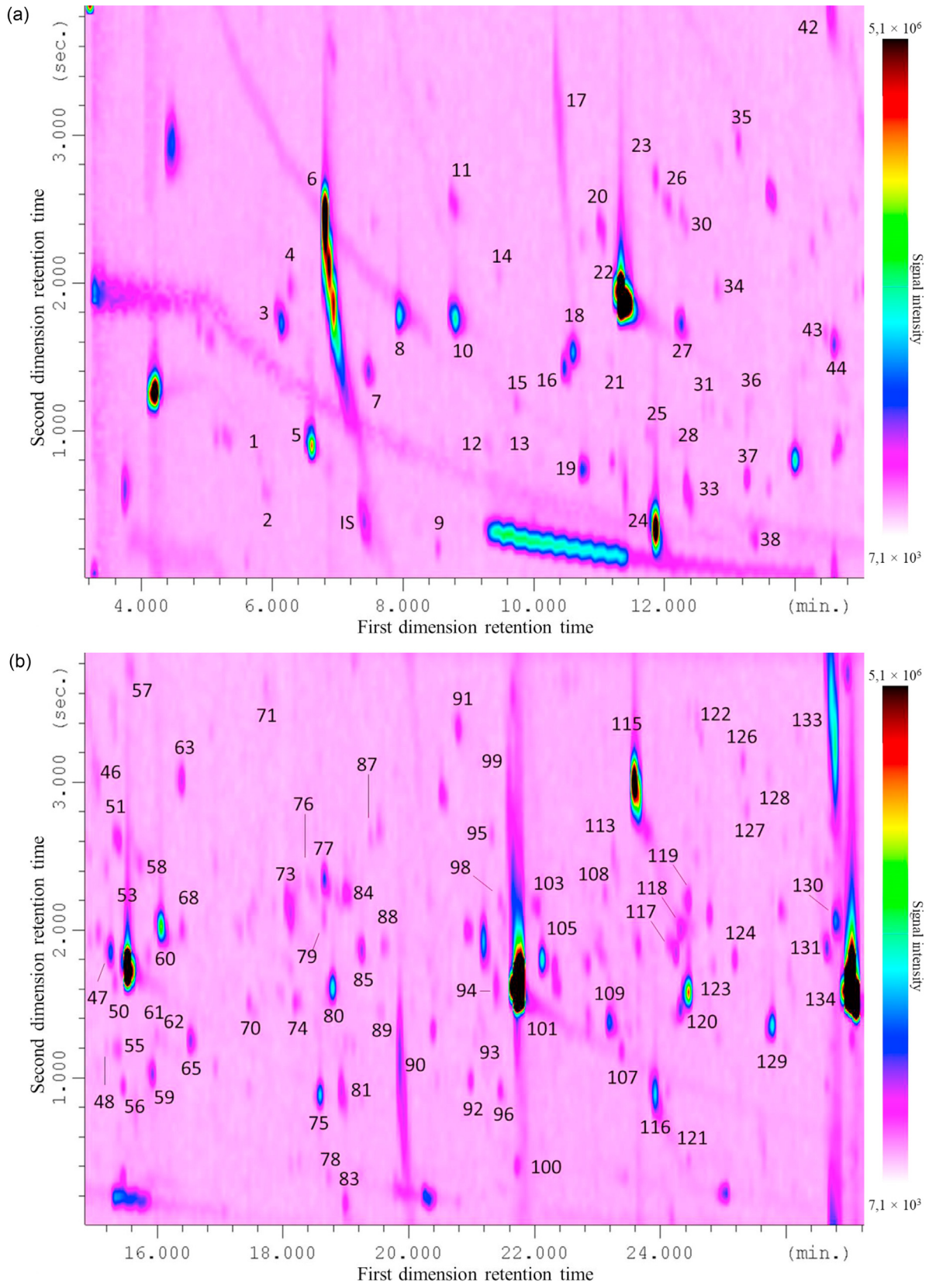

Fig. 6. Representative SPME Arrow / GC × GC–MS chromatogram of Blended Scotch whiskey. The three figures represent the retention time sections (a)–(c). Note that the retention time of the 1st dimension separation (x-axis) is given in minutes, that of the 2nd dimension separation (y-axis) in seconds.

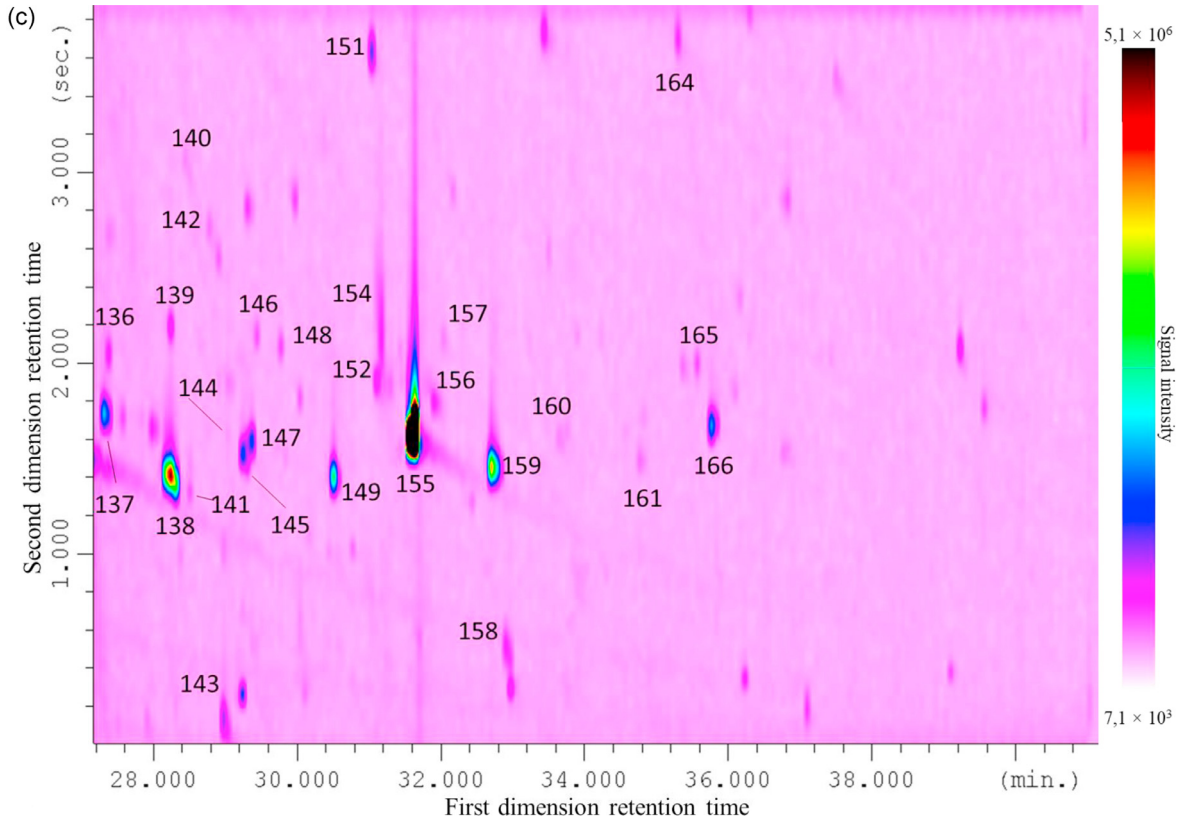

Fig. 6. Continued

with NaCl (30% w/v) and 70 µL of the ISTD working solution was added in the samples. Subsequently, the samples were closed with polytetrafluoroethylene (PTFE) coated silicone rubber septum aluminium caps. The extraction of the analytes was performed within 60 min at room temperature under constant stirring at 500 rpm, while desorption took place in the GC injection port for 2 min. After this time span, the SPME Arrow fiber remained in the injector for 10 more minutes for cleaning and was thus ready to be used for the next extraction.

The extraction conditions of the conventional SPME procedure were similar to those of the SPME Arrow procedure, to enable the comparison of the two techniques.

3. Results and discussion

3.1. Optimization of the SPME Arrow conditions

To ensure high method sensitivity, the main parameters that affect the extraction performance of the SPME Arrow method were thoroughly investigated and optimized using the one-variable-at-a-time (OVAT) approach. In this frame, the effect of the extraction time, the stirring rate and the salt content on the extraction efficiency were independently examined, while the remaining factors remained constant. Prior to each extraction, the whiskey samples were diluted to an ethanol content of 12% v/v, as suggested by Caldeira et al. [24] to minimize sensitivity loss for most VOCs and the sample pH was adjusted to 3.3. Adjusting the pH of the sample prior to the SPME procedure can enhance the sensitivity and selectivity for organic acids, which are present in whiskey samples [25]. An aliquot of 35 mL of the diluted whiskey sample was used for the SPME Arrow procedure [24]. With regard to the extraction temperature, no sample heating was employed and all extractions were carried out at ambient temperature from the sample headspace to avoid possible oxidative alteration of the volatiles pattern and to represent as closely as possible the authentic whiskey flavour [9].

The selection of the appropriate fiber coating plays a crucial role in the development of an SPME method. The chemical nature and the volatility of the target analytes in the investigated samples determines the type of coating used [26]. In this work, the semi-polar CAR/PDMS fibers were used for the extraction of the volatile compounds of the whiskey samples. This fiber has been previously reported to be an appropriate choice for the extraction of the VOCs from whiskey samples, showing good sensitivity towards hydrocarbons, monoterpenes, carbonyl compounds, higher alcohol acetates and isoamyl esters [24,26]. This extraction phase exhibits good sensitivity for smaller molecules, acids, esters and non-polar compounds and thus it serves as a good option for the extraction of a wide range of volatile flavour compounds [27]. It is assumed that the fibre coatings for the classical SPME and the SPME Arrow exhibit comparable properties and hence enrichment behavior, irrespective of the actual format.

During method optimization, all tests were carried out using the same whiskey sample (i.e., blended Scotch whiskey) for the reason of comparability. Six analytes from different chemical classes and consequently different chemical properties (i.e., volatility and polarity) were monitored during the optimization study. These compounds included two esters (i.e., octanoic acid ethyl ester and nonanoic acid ethyl ester), one carbonyl compound (i.e., 2-nonanone), one organic acid (i.e., dodecanoic acid) and two alcohols (i.e., 1-octanol and 1-decanol). Due to the different abundances of the monitored analytes, normalization of their peak areas was performed by dividing the peak area obtained under the examined conditions with their respective peak area under optimum/selected conditions.

Table 1
Comparative study of SPME Arrow and conventional SPME for the analysis of whiskey samples. The table reports the peak area values for those peaks that have been tentatively identified by their mass spectra and retention indices.

Nr.	Compound	LRI	Blended Scotch		Irish		Single malt Scotch	
			ARROW	Conv.	ARROW	Conv.	ARROW	Conv.
1	Heptane	700	73,034	-	63,179	-	-	-
2	3-Ethoxy-3-methyl-1-butene	700	189,651	-	971,281	-	-	-
3	Ethyl propanoate	708	3,682,418	644,764	2,812,5510	5,819,773	3,519,745	634,104
4	Propyl acetate	715	408,318	-	137,905	-	-	-
5	1,1-Diethoxyethane	721	1,422,4188	-	116,978,703	9,099,701	17,576,932	660,341
6	**3-Methyl-1-butanol**	733	256,740,608	45,053,251	426,900,243	59,850,519	1,050,679,561	120,195,386
7	Ethyl isobutyrate	752	1,661,640	-	1,203,447	136,227	-	-
8	Isobutyl acetate	768	7,806,245	776,523	1,177,424	-	5,527,551	-
9	Octane	800	213,278	-	941,190	62,249	327,235	-
10	Ethyl butanoate	803	9,581,642	1,416,587	5,927,820	742,485	13,154,245	2,061,469
11	Hexanal	805	847,079	220,643	2,050,915	1,118,996	-	-
12	1,1-Diethoxypropane	805	113,351	-	200,224	-	-	-
13	1-(1-Ethoxyethoxy)-propane	805	88,966	-	371,070	-	-	-
14	Butyl acetate	813	118,631	-	-	-	-	-
15	1-Ethoxy-3-methyl-but-2-ene	817	220,572	-	2,891,763	367,268	-	-
16	Ethyl-2-methylbutanoate	842	1,924,584	200,786	5,516,170	430,786	1,595,564	158,871
17	Furfural	845	970,286	275,377	458,982	177,450	2,419,673	1,726,130
18	Ethyl-3-methylbutanoate	850	3,944,547	532,723	8,322,583	663,528	3,388,896	758,566
19	1,1-Diethoxy-2-methyl-propane	851	2,522,513	235,489	2,330,023	153,047	2,120,634	418,175
20	Ethylbenzene	857	175,601	-	510,481	123,485	-	-
21	Isobutyl propionate	863	84,585	-	-	-	-	-
22	**Amyl acetate**	871	646,506,264	87,683,226	17,662,980	1,524,849	789,921,174	94,097,412
23	1-(1-Ethoxyethoxy)butane	872	297,147	-	882,779	-	259,240	73,988
24	Styrene	891	70,022,248	-	-	-	-	-
25	Acetyl valeryl	885	201,739	-	394,642	-	-	-
26	2-Heptanone	887	541,075	-	369,344	96,283	-	-
27	Ethyl pentanoate	889	2,646,562	304,844	3,308,238	341,939	1,415,508	188,182
28	3-Methyl-1-hexanol	896	624,101	-	1,253,761	-	3,079,270	169,610
29	1,3-Diethoxybutane	904	-	-	-	-	202,172	-
30	Heptanal	906	380,783	-	825,853	107,887	66,9864	420,468
31	*p*-Xylene	907	508,578	191,952	3,396,283	672,604	-	-
32	Heptan-2-ol	913	-	-	-	-	143,433	-
33	Furfuryl ethyl ether	917	1,434,986	427,767	-	-	-	-
34	Ethyl-2-methyl-2-butenoate	938	1,711,569	226,207	1,982,383	356,563	-	-
35	1,1-Diethoxy-2-propanone	941	490,305	-	2,923,117	356,778	-	-
36	1,1-Diethoxy-3-methyl-butane	946	6,572,391	645,396	8,770,094	721,220	1,551,749	-
37	α-pinene	948	644,324	-	-	-	-	-
38	3-Methyl-nonane	951	513,950	-	563,397	-	-	-
39	2-Methyl-1,3-pentanediol	959	-	-	2,262,277	185,386	-	-
40	1-Heptanol	960	-	-	-	-	1,033,854	80,747
41	Acetaldehyde ethyl-isoamyl-acetal	960	-	-	7,819,253	637,891	1,012,049	-
42	Benzaldehyde	960	2,321,991	1,204,577	2,707,909	1,253,642	2,392,095	1,927,031
43	1-Octen-3-ol	969	396,420	-	2,189,878	177,810	-	-
44	Pentyl propanoate	984	1,688,544	266,241	377,336	-	-	-
45	3-Octanone	986	-	-	121,816	-	3,452,251	333,375
46	6-Methyl-hept-5-en-2-one	986	117,136	-	-	-	-	-
47	2-Pentylfuran	991	2,380,969	470,475	132,070	11,4646	-	-
48	Myrcene	991	68,001	-	-	-	-	-
49	Ethyl-*(E)*-4-hexenoate	992	-	-	1,541,199	252,517	-	-
50	2,6-Dimethyl-2,4,6-octatriene	993	306,259	-	-	-	-	-
51	Isooctanol	995	339,480	-	-	-	-	-
52	Decane	1000	-	245,865	692,199	105,565	599,152	265,470
53	**Ethyl hexanoate**	1003	369,126,970	49,266,562	308,637,512	27,909,070	654,185,383	13,307,1449
54	*(S)*-2-Octanol	1004	-	-	294,376	-	357,653	-
55	1-(1-Ethoxyethoxy)-pentane	1004	929,777	-	-	-	698,921	654,919
56	1,1-Diethoxy pentane	1004	366,816	-	-	-	6,366,443	916,037
57	Dehydro-*cis*-linalool oxide	1006	363,538	-	78,204	-	271,163	60,941
58	Octanal	1006	440,951	83,225	-	-	-	-
59	3-Carene	1009	1,048,031	-	-	-	-	-
60	Hexyl acetate	1012	12,306,645	2,027,077	114,247	-	14,998,434	2,898,160
61	Isopentyl isobutyrate	1014	159,020	-	112,708	-	-	-
62	Benzofuran	1018	165,970	-	-	-	-	-
63	1,2,3-Trimethylbenzene	1020	1,154,270	761,385	1,480,107	922,615	718,317	560,294
64	2-Ethyl-1-hexanol	1030	-	-	229,674	143,596	-	-
65	Limonene	1030	765,378	438,193	1,000,981	143,801	723,842	591,419
66	2,2,6-Trimethyl-cyclohexanone	1035	-	-	190,798	-	-	-
67	Ethyl-hex-*(2E)*-enoate	1041	-	-	126,085	-	82,612	-
68	*p*-Cymene	1042	682,130	109,855	790,488	153,352	-	-
69	Ethyl-2-furoate	1053	-	-	724,476	184,940	-	-
70	Isopentyl butyrate	1054	331,099	-	205,783	-	-	-
71	2-Octenal	1058	100,440	-	126,316	-	-	-

(*continued on next page*)

Table 1 (*continued*)

Nr.	Compound	LRI	Blended Scotch		Irish		Single malt Scotch	
			ARROW	Conv.	ARROW	Conv.	ARROW	Conv.
72	Ethyl-4-methylhexanoate	1068	-	-	45,938,779	-	-	-
73	1-Octanol	1076	2,124,966	621,420	3,743,836	783,055	11,844,895	2,008,432
74	1,1,3-Triethoxypropane	1079	505,162	-	525,494	-	400,875	76,961
75	Hexanal diethyl acetal	1088	3,415,551	724,253	6,008,143	435,208	1,204,467	827,439
76	*(E)*-Hept-4-enoate	1091	153,613	-	2,501,447	434,619	325,664	64,717
77	2-Nonanone	1093	1,985,618	-	585,203	186,761	5,454,688	1,612,116
78	Propyl hexanoate	1096	108,711	-	-	-	-	-
79	Undecane	1100	204,490	-	-	-	-	-
80	Ethyl heptanoate	1101	5,460,882	1,577,250	16,152,079	3,067,650	7,388,299	2,232,399
81	Linalool	1101	1,374,987	-	-	-	6,251,635	1,069,449
82	3-Methylbutyl 2-methylbutanoate	1104	-	-	111,827	-	-	-
83	2-Nonanol	1105	487,331	134,603	1,046,514	445,572	621,552	-
84	Nonanal	1107	908,425	445,004	1,050,184	478,641	7,145,584	2,796,054
85	Heptyl acetate	1114	1,234,407	396,729	-	-	18,912,350	765,960
86	1,1,3-Triethoxybutane	1115	-	-	758,691	-	-	-
87	2-Ethyl-1,4-dimethyl-benzene	1119	260,604	-	-	-	-	-
88	Methyl octanoate	1125	317,363	135,217	225,873	97,083	293,265	242,326
89	Acetic acid, 2-ethylhexyl ester	1150	72,056	-	-	-	-	-
90	Ethyl benzoate	1160	6,420,703	4,445,586	-	-	-	-
91	*(E)*-2-Nonenal	1163	806,573	577,159	435,423	302,040	2,808,640	1,695,570
92	Linalool ethyl ether	1166	471,401	232,367	215,001	105,868	3,243,909	363,845
93	1-Nonanol	1176	121,760	-	1,136,036	324,909	1,197,768	139,854
94	Diethyl butanedioate	1183	897,646	217,340	1,838,957	510,407	8,185,114	1,452,982
95	Butyl hexanoate	1183	516,083	-	181,521	-	246,297	-
96	1,1-Diethoxy-Heptane	1190	391,776	212,168	572,073	237,643	1,528,553	1,097,424
97	3-Decanone	1190	-	-	-	-	471,828	202,562
98	*(Z)*-4-Octanoate	1191	292,691	146,313	3,576,155	1,424,090	579,307	232,318
99	Octanoic acid	1192	5,247,366	5,046,693	243,726	227,258	367,096	367,096
100	Dodecane	1200	491,936	388,723	1,194,340	765,292	1,341,827	740,272
101	**Ethyl octanoate**	1202	1,586,412,800	1,114,327,809	1,137,944,977	765,514,793	25,292,71,628	881,782,120
102	Ethyl-oct-*(2Z)*-enoate	1203	-	-	106,819	-	-	-
103	Decanal	1208	583,503	579,501	517,484	466,111	59,1550	346,170
104	Benzenecarboxylic acid	1213	-	-	1,580,340	996,999	7,111,540	4,664,672
105	Octyl acetate	1214	5,635,838	3,213,238	-	-	7,710,664	3,210,691
106	Ethyl-2-methyloctanoate	1218	-	-	524,954	306,725	-	-
107	1,3-*bis*(1,1-Dimethylethyl)-benzene	1249	777,128	6,233,912	3,229,506	1,860,017	-	-
108	Ethyl-oct-*(2E)*-enoate	1250	209,798	158,193	815,996	433,043	529,670	198,640
109	Isopentyl hexanoate	1252	3,269,618	1,834,900	803,975	383,459	4,707,274	1,902,631
110	2-Phenylethyl acetate	1259	-	-	-	-	250,282,814	99,953,771
111	*(Z)*-4-Decen-1-ol	1266	-	-	351,406	-	-	-
112	Ethyl benzeneacetate	1266	-	-	705,516	-	1,616,740	381,081
113	Isopropyl phenylacetate	1273	341,228	-	-	-	-	-
114	Citronellyl formate	1275	-	-	-	-	403,966	-
115	**Methyl 3-phenylpropionate**	1276	104,823,707	6,7891,244	-	-	160,716,786	-
116	1-Decanol	1278	6,837,285	4,070,032	4,458,914	2,376,529	31,309,218	9,903,634
117	Vitispirane	1286	1,200,514	817,574	2,180,266	1,581,599	-	-
118	3-Nonenoate	1290	1,161,833	782,295	2,558,468	1,428,374	1,132,083	450,811
119	2-Undecanone	1294	620,010	-	212,448	106,238	6,404,575	2,353,697
120	Ethyl nonanoate	1297	19,361,719	12,368,239	32,324,905	2,0328,920	25,977,921	11,187,761
121	Tridecane	1300	87,079	-	-	-	-	-
122	2-Undecanol	1303	144,167	-	-	-	-	-
123	Nonyl acetate	1313	147,747	111,004	-	-	210,016	105,679
124	Methyl decanoate	1327	574,098	416,983	428,587	305,472	769,175	301,298
125	β-Methyl-γ-octalactone	1344	-	-	2,474,887	804,813	939,055	263,066
126	Citronellyl acetate	1350	497,000	387,769	-	-	-	-
127	Ethyl-3-phenylpropionate	1359	247,167	171,528	-	-	-	-
128	*cis*-Geranyl acetate	1361	83,142	-	-	-	140,122	69,264
129	Butyl octanoate	1381	5,614,739	3,879,198	976,818	839,976	9,144,770	3,457,942
130	9-Decenoic acid	1386	4,127,094	3,247,172	14,805,595	11,169,603	24,674,526	18,044,741
131	*(Z)*-4-Decenoate	1389	1,703,448	1,301,762	3,784,060	2,978,127	-	-
132	1,2-Dihydro-1,1,6-trimethyl-naphthalene	1396	-	-	192,516	186,698	-	-
133	Decanoic acid	1398	49,808,638	25,278,501	20,431,211	15,686,902	137,475,276	22,340,012
134	**Ethyl decanoate**	1399	2,113,245,433	3,475,267,362	1,497,463,092	1,520,138,861	2,137,248,679	1,335,001,224
135	Tetradecane	1400	-	-	365,783	387,097	-	-
136	Dodecanal	1410	368,498	229,772	171,560	180,723	-	-
137	Decyl acetate	1412	4,455,537	3,135,322	-	-	4,240,662	1,815,455
138	3-Methylbutyl octanoate	1446	36,406,688	25,351,152	4,781,894	4,934,941	38,297,774	16,545,348
139	Ethyl-*trans*-2-decenoate	1447	588,635	425,400	904,613	824,999	952,380	387,768
140	*trans*-Geranylacetone	1450	134,860	-	117,608	-	223,443	174,269
141	*(E)*-β-Farnesene	1452	108,075	-	-	-	-	-
142	Methyl-undeca-*(2Z,4Z)*-dienoate	1470	137,982	96,899	-	-	-	-
143	1-Dodecanol	1476	1,518,159	1,108,395	604,838	463,566	-	-
144	Ethyl-undec-10-enoate	1485	88,516	-	170,534	228,980	-	-

(*continued on next page*)

Table 1 (*continued*)

Nr.	Compound	LRI	Blended Scotch		Irish		Single malt Scotch	
			ARROW	Conv.	ARROW	Conv.	ARROW	Conv.
145	Propyl decanoate	1487	2,485,987	1,482,612	2,474,762	-	3,263,535	1,047,891
146	Undecyl methyl ketone	1495	202,513	128,644	-	-	-	-
147	Ethyl-undecanoate	1498	2,792,411	144,5825	-	-	-	-
148	Tridecanal	1516	211,534	-	-	-	218,512	93,779
149	Isobutyl decanoate	1545	5,457,541	4,464,081	738,062	631,066	-	-
150	3,5-*bis*(1,1-Dimethylethyl)-phenol	1555	-	-	802,946	516,504	-	-
151	*(E)*-Nerolidol	1561	1,512,300	842,382	-	-	1,393,231	121,172
152	*cis*-5-Dodecenoic acid	1578	460,827	-	-	-	-	-
153	Methyl tridecanoate	1580	-	-	-	-	94,013	-
154	Dodecanoic Acid	1581	2,418,664	2,290,054	1,228,875	147,996	8,903,720	2,184,079
155	**Ethyl dodecanoate**	1598	588,572,307	585,470,244	370,531,682	309,865,147	731,681,168	354,099,250
156	Lauryl acetate	1610	533,326	453,090	-	-	606,016	263,360
157	Tetradecanal	1614	123,069	-	-	-	-	-
158	Phenethyl-hexanoate	1643	519,570	486,864	-	-	-	-
159	Isoamyl decanoate	1644	13,653,735	13,459,306	1,793,787	1,149,205	-	-
160	Propyl dodecanoate	1680	164,735	139,418	-	-	-	-
161	Isobutyl laurate	1744	197,222	151,408	-	-	-	-
162	Farnesyl acetate	1846	248,585	-	-	-	-	-
163	Isopentyl dodecanoate	1846	153,281	-	-	-	-	-
164	Phenylethyl-octanoate	1848	675,037	420,758	-	-	430,502	117,825
165	Ethyl-*(E)*-11-hexadecenoate	1986	672,880	-	-	-	-	-
166	Ethyl-9-hexadecenoate	1986	3,340,669	1,314,151	-	-	2,275,755	1,072,366
167	Ethyl hexadecanoate	1993	-	-	-	-	1,285,178	1,057,578

LRI: linear retention index
*Bold: most abundant compounds

3.1.1. Optimization of salt content

The salt content of the SPME Arrow procedure was investigated by adding different quantities of sodium chloride. Salt addition can reduce the solubility of the target analytes in the sample matrix, allowing them to be sorbed onto the fibre and thus resulting in enhanced extraction efficiency [28]. In this work, three different NaCl concentrations (i.e., 0, 15 and 30% w/v) were evaluated. Extraction of the target analytes took place within 45 min under constant stirring at 500 rpm. As shown in Fig. 1, sample saturation with 30% w/v NaCl resulted in increased extraction efficiency for most analytes (i.e., 2-nonanone, dodecanoic acid, 1-octanol and 1-decanol). Thus, further experiments were conducted using a NaCl content of 30% w/v.

3.1.2. Optimization of stirring rate

The stirring rate of the SPME procedure was also investigated. For this purpose, three different stirring rates (i.e., 250 rpm "weak stirring", 500 rpm "medium stirring" and 1000 rpm "intensive stirring") were evaluated. Sample agitation can enhance the extraction, especially for analytes with higher molecular mass [29]. The extraction of the target analytes was carried out for 45 min using a sample containing 30% w/v NaCl. Fig. 2 summarizes the results of the evaluation of the different stirring rates. As it can be observed, the extraction efficiency increased by increasing the stirring rate from 250 rpm to 500 rpm. However, a further increase up to 1000 rpm had a negative impact on the extraction efficiency. A likely explanation is that at higher stirring rates significantly more ethanol is transferred to the headspace, and may then compete with the other VOCs for the adsorption sites, because ethanol is present in whiskey at a concentration much higher than the aroma volatiles [30]. As a result, a stirring rate of 500 rpm was finally chosen.

3.1.3. Optimization of extraction time

Finally, the effect of the extraction time on the SPME Arrow method was investigated. Similarly to conventional SPME, it is important to find the optimum extraction time that ensures the extraction of the maximum amounts of analytes, leading to a high sensitivity [31]. In this study, extraction times were investigated between 15 and 60 min. As shown in Fig. 3, equilibrium was obtained at 30 min for nonanoic acid ethyl ester and at 45 min for 1-octanol. On the other hand, an increase of the extraction time up to 60 min has a positive impact on the extraction efficiency of 2-nonanone, dodecanoic acid, octanoic acid ethyl ester and 1-decanol. This observation can be attributed to the difference of volatility between the monitored analytes. An increase of the extraction time can enhance the extraction efficiency of compounds with high boiling point, while compounds with lower boiling point may remain unaffected as they reach equilibrium already after a shorter time [32]. Likewise, the equilibration time is also known to increase with an increasing fibre/headspace partition coefficient. Since adequate sensitivity was obtained at 60 min and to ensure an acceptable cycle time, an extraction time of 60 min was finally chosen.

3.2. Comparison of conventional SPME and SPME Arrow

The performance evaluation of the conventional SPME and SPME Arrow, under their respective optimum conditions, was carried out taking into consideration the total number of VOCs identified in different whiskey samples, as well as the sensitivity and the precision of the two techniques. Table 1 presents the VOCs that were identified in the whiskey samples by means of the SPME Arrow and a conventional SPME fiber of comparable enrichment phase. Values are reported as peak area results in this table, while the relative results, reported as area% are reported in the electronic supplementary material (Table S1).

As it can be observed, a total of 167 VOCs were identified for the three different varieties of whiskeys using the SPME Arrow, while only 121 VOCs were identified when the conventional SPME fiber was utilized. SPME Arrow enables the determination of compounds (e.g., 2-octenal, 3-ethoxy-3-methyl-1-butene, isopentyl-butyrate, heptan-2-ol, hexanoic acid butyl ester, etc.) that are present in whiskey samples, even though their identification under the same experimental conditions was not possible when conventional SPME was used.

Accordingly, SPME Arrow and conventional SPME were compared in terms of their overall sensitivity. For this purpose, a

Table 2
Analysis of whiskey samples by SPME Arrow combined with GC × GC–MS, expressed as the normalised peak area ratio normalized to the internal standard, 3-methyl-3-pentanol.

Nr.	Compounds	Blended Scotch [rel. intensity±SD]	Irish [rel. intensity±SD]	Single malt Scotch [rel. intensity±SD]
1	Heptane	0.064 ± 0.002	0.123 ± 0.048	0.066 ± 0.002
2	3-Ethoxy-3-methyl-1-butene	0.032 ± 0.002	0.138 ± 0.028	-
3	Ethyl propanoate	0.628 ± 0.190	1.126 ± 0.065	0.384 ± 0.024
4	Propyl acetate	0.069 ± 0.014	0.021 ± 0.005	-
5	1,1-Diethoxyethane	2.421 ± 0.562	16.835 ± 1.144	1.643 ± 0.384
6	3-Methyl-1-butanol	43.691 ± 8.662	60.641 ± 10.994	101.135 ± 13.937
7	Ethyl-isobutyrate	0.033 ± 0.002	-	-
8	Isobutyl acetate	109.605 ± 4.324	2.557 ± 0.303	128.651 ± 8.109
9	Octane	0.075 ± 0.020	-	-
10	Ethyl butanoate	0.669 ± 0.061	1.192 ± 0.166	0.341 ± 0.080
11	Hexanal	-	-	0.126 ± 0.002
12	1-(1-Ethoxyethoxy)-propane	0.015 ± 0.004	0.053 ± 0.012	-
13	1,1-Diethoxy-propane	0.019 ± 0.004	0.030 ± 0.003	-
14	Butyl acetate	0.020 ± 0.003	-	-
15	1-Ethoxy-3-methyl-but-2-ene	-	0.366 ± 0.040	0.093 ± 0.009
16	Ethyl-2-methylbutanoate	-	0.018 ± 0.002	-
17	Furfural	0.042 ± 0.004	-	-
18	Ethyl 3-methylbutanoate	0.327 ± 0.035	0.802 ± 0.140	0.156 ± 0.002
19	1,1-Diethoxy-2-methyl-propane	0.428 ± 0.037	0.346 ± 0.037	0.207 ± 0.007
20	Ethylbenzene	0.100 ± 0.013	0.136 ± 0.024	0.095 ± 0.014
21	Isobutyl propionate	0.014 ± 0.004	-	-
22	Amyl acetate	62.590 ± 2.991	45.002 ± 3.974	64.357 ± 1.385
23	1-(1-Ethoxyethoxy)butane	0.050 ± 0.008	0.131 ± 0.020	0.027 ± 0.008
24	Acetyl valeryl	0.034 ± 0.001	0.059 ± 0.008	-
25	2-Heptanone	0.092 ± 0.003	0.054 ± 0.006	-
26	Ethyl pentanoate	0.062 ± 0.010	-	0.701 ± 0.190
27	Styrene	11.871 ± 0.476	-	-
28	3-Methyl-1-hexanol	0.105 ± 0.022	0.182 ± 0.023	0.307 ± 0.045
29	1,3-Diethoxybutane	-	-	0.022 ± 0.008
30	Heptanal	-	-	0.015 ± 0.005
31	-Xylene	0.086 ± 0.009	0.422 ± 0.025	-
32	Heptan-2-ol	0.020 ± 0.006	-	-
33	Furfuryl ethyl ether	0.164 ± 0.014	0.079 ± 0.014	0.234 ± 0.035
34	Ethyl-2-methyl-2-butenoate	0.290 ± 0.006	0.288 ± 0.082	-
35	1,1-Diethoxy-2-propanone	0.083 ± 0.017	0.452 ± 0.065	-
36	1,1-Diethoxy-3-methyl-butane	0.037 ± 0.009	0.417 ± 0.065	-
37	α-pinene	0.058 ± 0.001	-	-
38	3-Methyl-nonane	0.155 ± 0.031	0.154 ± 0.024	0.706 ± 0.040
39	2-Methyl-1.3-pentanediol	-	0.329 ± 0.056	-
40	1-Heptanol	-	-	0.109 ± 0.072
41	Acetaldehyde ethyl-isoamyl acetal	-	1.194 ± 0.333	0.109 ± 0.095
42	Benzaldehyde	0.110 ± 0.029	-	-
43	1-Octen-3-ol	0.067 ± 0.005	0.322 ± 0.012	-
44	Pentyl propanoate	0.286 ± 0.006	0.056 ± 0.005	-
45	3-Octanone	-	0.017 ± 0.001	0.348 ± 0.087
46	6-Methyl-hept-5-en-2-one	0.023 ± 0.013	0.016 ± 0.001	0.023 ± 0.006
47	2-Pentylfuran	0.405 ± 0.066	0.020 ± 0.003	-
48	Myrcene	0.062 ± 0.004	0.012 ± 0.003	0.027 ± 0.006
49	Ethyl *(E)*-4-hexenoate	-	0.021 ± 0.008	0.008 ± 0.001
50	2,6-Dimethyl-2,4,6-octatriene	0.052 ± 0.014	-	-
51	Isooctanol	0.027 ± 0.002	0.017 ± 0.009	-
52	Decane	0.099 ± 0.002	0.075 ± 0.008	0.066 ± 0.006
53	Ethyl hexanoate	0.087 ± 0.002	0.025 ± 0.001	0.023 ± 0.008
54	*(S)*-2-Octanol	-	0.045 ± 0.003	0.037 ± 0.015
55	1-(1-Ethoxyethoxy)-pentane	0.116 ± 0.023	0.120 ± 0.058	-
56	1,1-Diethoxy-pentane	0.158 ± 0.028	-	0.069 ± 0.004
57	Dehydro-*cis*-linalool oxide	0.080 ± 0.008	0.032 ± 0.008	0.370 ± 0.489
58	Octanal	-	0.016 ± 0.008	-
59	3-Carene	0.178 ± 0.043	-	-
60	Hexyl acetate	2.088 ± 0.140	0.018 ± 0.003	1.445 ± 0.253
61	Isopentyl Isobutyrate	0.283 ± 0.061	0.170 ± 0.017	-
62	Benzofuran	17.806 ± 2.443	-	16.057 ± 2.678
63	1,2,3-Trimethyl-benzene	0.393 ± 0.006	0.427 ± 0.038	0.232 ± 0.019
64	2-Ethyl-1-hexanol	-	0.074 ± 0.010	-
65	Limonene	0.091 ± 0.007	-	0.061 ± 0.015
66	2,2,6-Trimethyl-cyclohexanone	-	-	0.039 ± 0.003
67	Ethyl-hex-*(2E)*-enoate	0.927 ± 0.080	2.358 ± 0.248	0.723 ± 0.018
68	*p*-Cymene	269.170 ± 21.681	168.624 ± 15.551	250.604 ± 22.265
69	2-Ethyl-furoate	0.243 ± 0.007	-	-
70	Isopentyl butyrate	0.952 ± 0.021	0.149 ± 0.048	1.186 ± 0.210
71	2-Octenal	0.017 ± 0.001	0.019 ± 0.002	-
72	Ethyl-4-methylhexanoate	-	6.678 ± 0.976	-

(*continued on next page*)

Table 2 (*continued*)

Nr.	Compounds	Blended Scotch [rel. intensity±SD]	Irish [rel. intensity±SD]	Single malt Scotch [rel. intensity±SD]
73	1-Octanol	0.360 ± 0.010	0.544 ± 0.060	1.193 ± 0.288
74	1,1,3-Triethoxy-propane	0.086 ± 0.002	0.082 ± 0.003	0.039 ± 0.001
75	Hexanal diethyl acetal	0.144 ± 0.006	0.292 ± 0.029	-
76	*(E)*-Hept-4-enoate	0.026 ± 0.001	0.364 ± 0.010	0.032 ± 0.003
77	2-Nonanone	0.337 ± 0.014	0.085 ± 0.006	0.534 ± 0.013
78	Propyl hexanoate	0.088 ± 0.009	-	-
79	Undecane	0.034 ± 0.010	-	-
80	Ethyl heptanoate	0.067 ± 0.025	0.087 ± 0.026	0.148 ± 0.018
81	Linalool	0.133 ± 0.015	0.148 ± 0.016	0.072 ± 0.013
82	3-Methylbutyl 2-methylbutanoate	0.153 ± 0.026	0.272 ± 0.026	0.822 ± 0.076
83	2-Nonanol	0.083 ± 0.019	0.171 ± 0.023	0.062 ± 0.011
84	Nonanal	0.257 ± 0.019	-	0.132 ± 0.042
85	Heptyl acetate	0.210 ± 0.025	-	1.681 ± 1.666
86	1,1,3-Triethoxybutane	-	0.117 ± 0.017	-
87	2-Ethyl-1,4-dimethyl-benzene	0.131 ± 0.012	0.476 ± 0.075	-
88	Methyl octanoate	0.889 ± 0.022	0.041 ± 0.004	0.037 ± 0.008
89	Acetic acid, 2-ethylhexyl ester	0.012 ± 0.001	-	-
90	Ethyl benzoate	0.028 ± 0.005	-	-
91	*(E)*-2-Nonenal	0.137 ± 0.002	0.067 ± 0.004	0.283 ± 0.068
92	Linalool ethyl ether	0.233 ± 0.004	-	0.627 ± 0.126
93	1-Nonanol	0.021 ± 0.001	0.163 ± 0.027	0.120 ± 0.026
94	Diethyl butanedioate	1.115 ± 0.104	1.332 ± 0.273	0.154 ± 0.018
95	Butyl hexanoate	0.018 ± 0.001	-	-
96	1,1-Diethoxy-Heptane	0.012 ± 0.001	0.009 ± 0.001	-
97	3-Decanone	-	-	0.047 ± 0.011
98	*(Z)*-4-Octanoate	0.049 ± 0.005	0.530 ± 0.047	0.057 ± 0.000
99	Octanoic acid	0.114 ± 0.004	-	0.043 ± 0.008
100	Dodecane	0.063 ± 0.005	0.026 ± 0.009	-
101	Ethyl octanoate	6.179 ± 0.587	0.712 ± 0.091	3.821 ± 0.529
102	Ethyl-oct-*(2Z)*-enoate	0.036 ± 0.010	0.122 ± 0.053	0.052 ± 0.002
103	Decanal	-	0.029 ± 0.003	-
104	Benzenecarboxylic acid	-	0.102 ± 0.028	0.163 ± 0.031
105	Octyl acetate	0.956 ± 0.075	-	0.768 ± 0.104
106	Ethyl-2-methyloctanoate	0.054 ± 0.003	0.032 ± 0.008	0.028 ± 0.006
107	1,3-*bis*(1,1-Dimethylethyl)-benzene	0.196 ± 0.014	0.220 ± 0.048	0.071 ± 0.003
108	Ethyl-oct-*(2E)*-enoate	3.282 ± 0.095	4.938 ± 0.632	2.593 ± 0.411
109	Isopentyl hexanoate	0.058 ± 0.002	-	-
110	2-Phenylethyl acetate	-	-	24.983 ± 3.960
111	*(Z)*-4-Decen-1-ol	-	0.052 ± 0.009	-
112	Ethyl benzeneacetate	0.044 ± 0.008	-	-
113	Isopropyl phenylacetate	-	0.116 ± 0.029	-
114	Citronellyl formate	0.084 ± 0.005	-	-
115	Methyl-3-phenylpropionate	0.030 ± 0.006	-	-
116	1-Decanol	1.159 ± 0.061	0.648 ± 0.093	3.142 ± 0.251
117	Vitispirane	0.204 ± 0.017	0.469 ± 0.073	-
118	3-Nonenoate	0.197 ± 0.009	0.379 ± 0.049	0.110 ± 0.010
119	2-Undecanone	0.105 ± 0.001	0.032 ± 0.005	0.657 ± 0.270
120	Ethyl nonanoate	0.087 ± 0.022	0.079 ± 0.010	-
121	Tridecane	0.015 ± 0.001	-	-
122	2-Undecanol	0.024 ± 0.005	-	-
123	Nonyl acetate	0.025 ± 0.005	-	0.021 ± 0.007
124	Methyl decanoate	358.434 ± 22.560	224.226 ± 18.354	214.390 ± 23.777
125	β-methyl-γ-octalactone	1.090 ± 0.120	-	-
126	Citronellyl acetate	0.078 ± 0.016	-	-
127	Ethyl-3-phenylpropionate	0.114 ± 0.014	-	-
128	*cis*-Geranyl acetate	-	0.110 ± 0.013	-
129	Butyl octanoate	1.628 ± 0.238	0.849 ± 0.089	0.759 ± 0.394
130	9-Decenoic acid	0.701 ± 0.073	2.218 ± 0.077	2.121 ± 2.853
131	*(Z)*-4-Decenoate	0.289 ± 0.012	0.568 ± 0.255	-
132	1,2-Dihydro-1,1,6-trimethyl-naphthalene	0.012 ± 0.001	-	-
133	Decanoic acid	0.931 ± 0.110	0.105 ± 0.020	-
134	Ethyl decanoate	8.450 ± 0.632	2.999 ± 0.127	13.740 ± 2.339
135	Tetradecane	-	0.052 ± 0.006	-
136	Dodecanal	0.409 ± 0.097	0.362 ± 0.017	0.308 ± 0.021
137	Decyl acetato	0.756 ± 0.049	-	0.405 ± 0.099
138	3-Methylbutyl octanoate	-	0.078 ± 0.019	-
139	Ethyl-*trans*-2-decenoate	-	-	0.226 ± 0.026
140	*trans*-Geranylacetone	0.014 ± 0.004	-	0.014 ± 0.002
141	*(E)*-β-Farnesene	0.030 ± 0.005	0.072 ± 0.012	-
142	Methyl-undeca-*(2Z,4Z)*-dienoate	0.023 ± 0.005	-	-
143	1-Dodecanol	0.257 ± 0.029	0.090 ± 0.015	-
144	Ethyl-undec-10-enoate	0.015 ± 0.003	0.024 ± 0.003	-
145	Propyl decanoate	0.097 ± 0.011	0.065 ± 0.011	0.076 ± 0.009

(*continued on next page*)

Table 2 (*continued*)

Nr.	Compounds	Blended Scotch [rel. intensity±SD]	Irish [rel. intensity±SD]	Single malt Scotch [rel. intensity±SD]
146	Undecyl methyl ketone	0.034 ± 0.001	-	-
147	Ethyl-undecanoate	0.473 ± 0.021	-	-
148	Tridecanal	0.036 ± 0.004	-	0.021 ± 0.001
149	Isobutyl decanoate	2.416 ± 0.301	0.251 ± 0.024	-
150	3,5-*bis*(1,1-Dimethylethyl)-phenol	0.449 ± 0.035	0.478 ± 0.125	0.138 ± 0.011
151	*(E)*-Nerolidol	-	0.031 ± 0.012	-
152	*cis*-5-Dodecenoic acid	0.056 ± 0.007	0.031 ± 0.007	-
153	Methyl tridecanoate	-	-	0.009 ± 0.001
154	Dodecanoic Acid	0.026 ± 0.008	-	-
155	Ethyl dodecanoate	0.410 ± 0.034	0.155 ± 0.030	0.879 ± 0.052
156	Lauryl acetate	0.554 ± 0.020	0.121 ± 0.014	0.464 ± 0.014
157	Tetradecanal	0.021 ± 0.001	-	-
158	Phenethyl-hexanoate	0.581 ± 0.091	0.914 ± 0.169	0.114 ± 0.012
159	Isoamyl decanoate	-	0.104 ± 0.049	0.059 ± 0.003
160	Propyl dodecanoate	99.991 ± 14.485	52.700 ± 9.882	72.068 ± 2.373
161	Isobutyl laurate	1.327 ± 0.233	0.163 ± 0.007	0.558 ± 0.148
162	Farnesyl acetate	0.018 ± 0.001	-	-
163	Isopentyl dodecanoate	0.084 ± 0.016	0.183 ± 0.027	0.136 ± 0.043
164	Phenylethyl-octanoate	0.036 ± 0.007	0.131 ± 0.008	0.033 ± 0.007
165	Ethyl *(E)*-11-hexadecenoate	0.028 ± 0.013	-	-
166	Ethyl-9-hexadecenoate	0.042 ± 0.001	-	-
167	Ethyl hexadecanoate	-	0.226 ± 0.035	-

blended Scotch whiskey sample was analyzed in three repetitions and the comparison of the two techniques was carried out in terms of the obtained areas for selected compounds. As shown in Fig. 4, the sensitivity of the determination for the VOCs is considerably higher when the sample is extracted with the use of SPME Arrow fiber for all the determined classes of compounds. Enhancement factors are calculated as the peak area ratio of the SPME Arrow measurement in relation to the conventional SPME measurement of individual compounds in the same sample. Individual enhancement factors have been grouped and averaged according to compound class to be more representative. The utilization of SPME Arrow resulted in sensitivity enhancement factors of up to 6.1. These results are in accordance with previous studies that reported the superiority of SPME Arrow in terms of method sensitivity [6,33].

Finally, the repeatability of SPME Arrow and conventional SPME were compared on the basis of average relative standard deviation (RSD) values for the peak areas. The data were obtained from the triplicate analysis of the blended Scotch whiskey sample. Fig. 5 presents the results for the two techniques, according to chemical compound class. The higher precision as well as the greater sensitivity of the SPME Arrow fiber is attributed to the greater amount of sorptive phase and the greater surface area compared to the conventional SPME fiber, and the consequently resulting larger peak areas in most cases [14].

As it can be observed, the utilization of SPME Arrow fibers leads to more reproducible results in comparison with conventional SPME fibers. All things considered, the use of the SPME Arrow technique brings considerable advantages over conventional SPME technique since it enables the extraction of a higher number of total compounds, as well as higher sensitivity and reproducibility.

3.3. Application of SPME Arrow for the determination of VOCs in whiskey samples

As proof-of-concept, the optimized SPME Arrow method was employed for the extraction and preconcentration of VOCs from different types of whiskey samples prior to their determination by GC × GC–MS. Unequivocally, Irish whiskey and Scotch whiskey are among the most famous whiskey types. Scotch whiskey is produced and matured in oak casks for at least three years in Scottish distilleries located in specific designated regions. This type of whiskey includes five distinct categories, i.e., single malt Scotch whiskey, single grain Scotch whiskey, blended Scotch whiskey, blended malt Scotch whiskey and blended grain Scotch whiskey. Irish whiskey is another type of distilled beverage internationally recognised by Geographical Indication and it is produced from either malted barley or a mixture of unmalted and malted other cereals and barley. In the latter case, the minimum content of malted barley is 25% [34]. Many of the VOCs that are expected to be determined in whiskey samples are common to different whiskeys but differ analytically in terms of the relative amount [24]. In Fig. 6, three expansions of a representative chromatogram of a Blended Scotch whiskey sample are shown.

Moreover, Table 2 summarizes the results from all samples. The semi-quantitative analysis of the concentration ranges for the VOCs in all samples was conducted by comparing the peak area of each analyte to the peak area of the internal standard (ISTD) 3-methyl-3-pentanol.

Fatty acid esters comprise a significant group of VOCs in whiskey samples. These compounds exhibit a pleasant odour and some of them have a high odour impact and as a result they play an important role as aroma components of whiskey samples. Short-chain fatty acid esters including ethyl-, isobutyl- and 3-methylbutyl esters are common constituents of whiskey samples and their presence is associated with a pleasant aroma [35]. For example, isoamyl acetate and ethyl hexanoate are compounds with fruity aromas, while 2-phenylethyl acetate exhibits floral aroma [4]. Other esters that are determined in whiskey samples in significant amounts are the ones of octanoic, decanoic and dodecanoic acids, while ethyl *E*-11-hexadecenoate is a common compound that is mainly found in Scotch whiskeys [35].

Furanic compounds that were detected in the whiskey samples included 2-pentylfuran and furfural. Furfural exhibits a roasty aroma described as “baked/toasted almond”. 2-pentylfuran exhibits an earthy aroma, described as “gas/bad smell” and “stable”, respectively. Among the major alcohols that were detected in the whiskey samples, most of the detected VOCs (i.e., 3-methyl-1-butanol or isoamyl alcohol) exhibit a fatty odour type [4].

A wide range of aldehydes with diverse odour type were also determined in the whiskey samples. Among them, compounds with vegetal [e.g., (E)-2-octenal described as “vegetable/cabbage” and hexanal described as “green/vegetative”], chemical (e.g., nonanal described as “soap/fresh”), fatty [e.g., (E)-2-nonenal de-

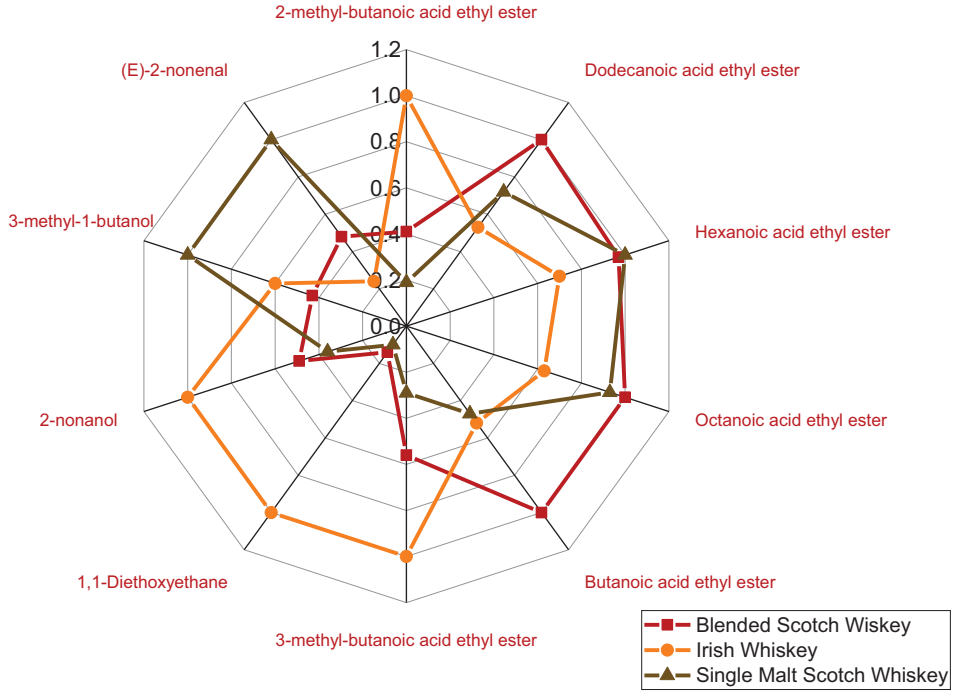

Fig. 7. Comparison of key odorants VOCs in three different whiskey samples in the form of a spider plot. In this plot, the individual rays represent the relative concentration of each key odorant in the three whiskey varieties, normalized to the whiskey type that has highest concentration of each compound.

scribed as "fried/toasted/fatty"] and grassy aromas (e.g., heptanal described as "seaweed/grass/rubber" and decanal described as "grass/lemon") were found in the whiskey samples [4,36]. In contrast to this, the presence of styrene can be attributed to sample contamination [37]. Alcoholic beverages are known to be good extractants for polystyrene from packaging materials [38]. In the current case, the polymer liner of the screw cap is suspected to be the source of the observed contamination.

The evaluation of the differences between different types of whiskey by means of SPME Arrow was also investigated. Fig. 7 shows three spider plots providing the comparison of the intensity of ten VOCs that have been identified as key odorant compounds and that were tentatively identified in the whiskey samples (i.e., 1,1-diethoxyethane, 3-methyl-1-butanol, 2-nonanol, (E)-2-nonenal, dodecanoic acid ethyl ester, octanoic acid ethyl ester, hexanoic acid ethyl ester, butanoic acid ethyl ester, 3-methyl-butanoic acid ethyl ester and 2-methyl-butanoic acid ethyl ester) [39–41]. The concentration of each compound was normalized to the highest concentration found for the respective compound among the three different Whiskey samples. The relative concentration of each compound was plotted along the rays of this spider diagram with a span of 0-10, representing 0–100% of the maximum concentration. As it can be observed, relatively high differences were observed between the particular whiskey types that were analyzed in this study. Thus, SPME Arrow could potentially serve as a simple and efficient extraction technique for the differentiation of different types of whiskey samples.

4. Conclusions

In this work, the SPME Arrow technique combined with GC × GC-MS was evaluated for the first time for the sampling of VOCs of different types of whiskey samples. The main parameters affecting the performance of the SPME Arrow protocol were investigated and optimized and the proposed method was compared with the procedure using conventional SPME fibers. Under optimum conditions, the utilization of the SPME Arrow fibers resulted in better sensitivity and repeatability compared to conventional CAR/PDMS fibers. Moreover, the utilization of the SPME Arrow technique enabled the detection of more volatile constituents compared to the conventional SPME format. It can thus be concluded that the coupling of SPME–Arrow and GC × GC-MS results in a powerful analytical workflow that provides more comprehensive information compared to already existing sample preparation techniques, making it most appropriate for hunting molecules in complex samples.

Declaration of Competing Interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Antonio Ferracane: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Natalia Manousi:** Investigation, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Peter Q. Tranchida:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **George A. Zachariadis:** Conceptualization, Project administration, Supervision, Writing – review & editing. **Luigi Mondello:** Conceptualization, Project administration, Supervision, Writing – review & editing. **Erwin Rosenberg:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Resources, Writing – review & editing.

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Supplementary materials

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